DEPARTMENT OF HEALTH AND HUMAN SERVICES

MEMORANDUM OF CONFERENCE
September 19, 1994

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Subject: Glyphosate-tolerant soybeans.

Keywords: Soybean; glyphosate (herbicide) tolerance; EPSPS from *Agrobacterium sp.* strain CP4 (CP4 EPSPS).

This meeting was intended to bring Monsanto's consultation with FDA on the food and feed safety of this product to closure. Monsanto had previously met with the agency on

this subject (see memorandum of June 24, 1993 meeting in Subject File 1319 (SBJ 1319)).

Intended Effect and Food/Feed Use

The intended effect of this genetic modification is to render soybean *(Glycine max)* plants tolerant to commercially relevant levels of the non-selective herbicide glyphosate. Soybeans or processed products derived from soybeans are used for both human and animal food, with the large majority being used in animal feed.

Mechanism of Intended Effect

Glyphosate's herbicidal activity is conferred by its ability to potently inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which has an essential function in all plants, fungi, and bacteria in the biosynthesis of aromatic amino acids. Monsanto has isolated a gene from the soil bacterium *Agrobacterium sp.* strain CP4 which encodes an EPSPS (hereafter referred to as CP4 EPSPS) which is highly resistant to inhibition by glyphosate. Expression of relatively low levels of CP4 EPSPS renders soybeans tolerant of commercially relevant levels of glyphosate.

Molecular Alterations and Characterization

A map of the pUC119-based vector used for particle gun-mediated transformation of the CP4 EPSPS expression cassette is shown on page 21 of Monsanto's submission of September 2, 1994. Based on PCR analysis and restriction mapping of genomic DNA from the final transgenic line intended for commercialization (line 40-3-2, derived from the parental line A5403), Monsanto has concluded that the inserted DNA spans a maximum of 2280 base pairs (bp) in length and extends from a breakpoint within the enhanced cauliflower mosaic virus 35S (E35S) promoter to a breakpoint 3' of the poly-A site of the nopaline synthase (NOS) transcriptional terminator (refer to page 24 of the submission). This transgene is predicted to express a chimeric primary translation product comprised of an N-terminal petunia chloroplast transit peptide (CTP) fused to the full-length CP4 EPSPS coding sequence. The CTP is intended to target the mature CP4 EPSPS polypeptide to its site of action in the chloroplast.

Based on genomic restriction mapping and genetic analysis, Monsanto has concluded that the CP4 EPSPS-expressing transgene is present in one copy, is integrated at a single locus, segregates as a single dominant Mendelian trait, and is molecularly stable over six generations. Monsanto also stated that the trait is phenotypically stable over several generations.

Monsanto has concluded that other sequences present on the original vector (including the *kan*^{r} gene, the gene encoding *E. coli* glucuronidase, a second CP4 EPSPS expression

cassette, and sequences derived from the pUC119 parent vector - refer to page 21 of the submission) were not present in line 40-3-2 as judged by Southern analysis.

Safety of the Expressed Protein

According to Monsanto, based on the N-terminal sequence of purified soybean CP4 EPSPS, the CTP, as expected, is cleaved from the primary translation product upon transport into the chloroplast (or plastid stroma), leaving the mature CP4 EPSPS protein. CP4 EPSPS is similar in predicted amino acid sequence to EPSPS enzymes from a wide variety of prokaryotic and eukaryotic organisms. Based on comparisons with known protein allergens and toxins carried out by standard methods, Monsanto has concluded that CP4 EPSPS is not significantly similar in amino acid sequence to known protein toxins or allergens. Monsanto also reported that soybeans subjected to heat treatment which is typical of that experienced during normal soybean processing show no detectable EPSPS activity, indicating that typical soybean processing completely destroys both CP4 EPSPS and endogenous soybean EPSPS activity. Virtually all soy products used in food or feed are heat-processed prior to consumption.

In order to produce sufficient material for safety and metabolism studies, Monsanto has produced CP4 EPSPS in *E. coli*. Based on comparisons of molecular weight, N-terminal sequence, specific activity, immunoreactivity, and the absence of glycosylation, Monsanto has concluded that *E. coli*-produced CP4 EPSPS is equivalent to CP4 EPSPS purified from soybeans. According to Monsanto, CP4 EPSPS is rapidly digested in simulated gastric and intestinal fluid and, as expected, showed no acute toxicity in a mouse gavage study. Monsanto also stated that CP4 EPSPS does not fit the profile of the typical allergen because 1) it is not heat stable; 2) it is not a major protein in soybeans; 3) it is not resistant to digestion; and 4) it is not glycosylated.

Compositional Analysis

Based on the nature of the genetic modification, it was not expected that glyphosate-tolerant soybeans would differ compositionally from other soybean varieties. To confirm this expectation, Monsanto carried out compositional analyses which focussed on analysis of whole beans but also included compositional analyses of major soy-derived products, including toasted soybean meal, soy oil, defatted soy flour, and soy protein isolate.

Based on their analysis of whole beans (and, for certain parameters, various soy products), Monsanto has concluded that glyphosate-tolerant soybeans are not significantly different from other soybean varieties in protein, fat, fiber, ash, carbohydrate, amino acid, fatty acid, trypsin inhibitor, lectin, isoflavone (genistein and daidzen), phospholipid

(lecithin), phytate, stachyose, or raffinose content. Monsanto's analysis of some of these parameters in glyphosate-treated soybeans led them to similar conclusions.

In order to verify that glyphosate-tolerant varieties of soybeans express levels of soybean allergens no greater than traditional varieties, Monsanto carried out immunoblot analysis using pooled serum from individuals allergic to soybeans. Monsanto reported that there was no difference between glyphosate-tolerant and control lines of soybeans in the level of expression of immunoreactive material.

Wholesomeness Studies

Monsanto described the results of wholesomeness studies they carried out in rats, chickens, catfish, dairy cattle, and bobwhite quail. On the basis of their consideration of the totality of these studies, Monsanto has concluded that there is no significant difference in the wholesomeness of glyphosate-tolerant and traditional soybean varieties, as expected from their compositional analysis. These data are summarized on page 49 of Monsanto's September 2 submission.

Conclusions

Monsanto has concluded, in essence, that the glyphosate-tolerant soybean variety they have developed is not significantly altered within the meaning of 21 CFR 170.30(f)(2) when compared to soybean varieties with a history of safe use. At this time, based on Monsanto's description of its data and analysis, the agency considers Monsanto's consultation on this product to be complete.

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